

Ribosomal Protein Binding During Ribosomal RNA Maturation

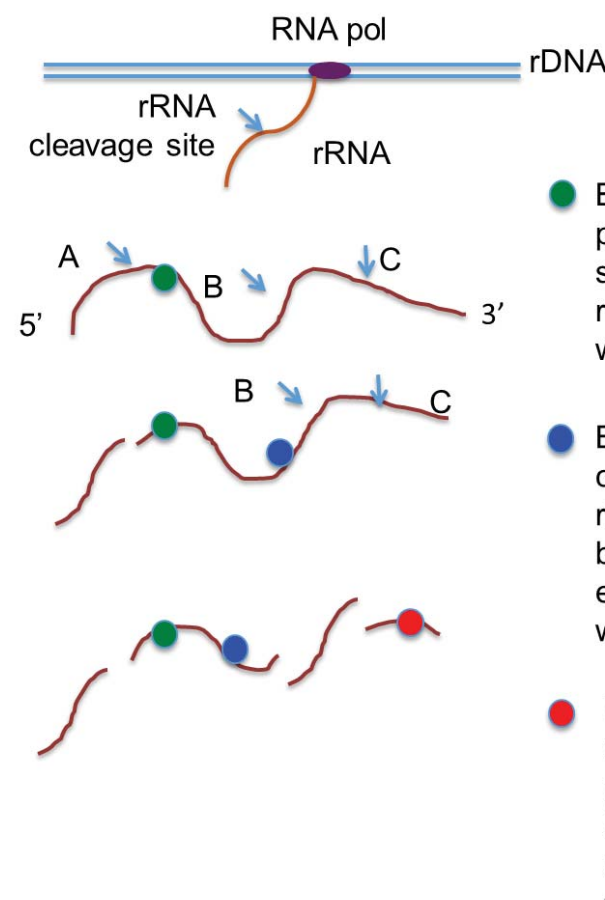
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INTRODUCTION

Ribosomal RNA (rRNA) and ribosomal proteins are integral parts of the ribosome complex. Formation of these particles involves extensive synthesis in which rRNA and ribosomal proteins join together to form mature ribosomes.

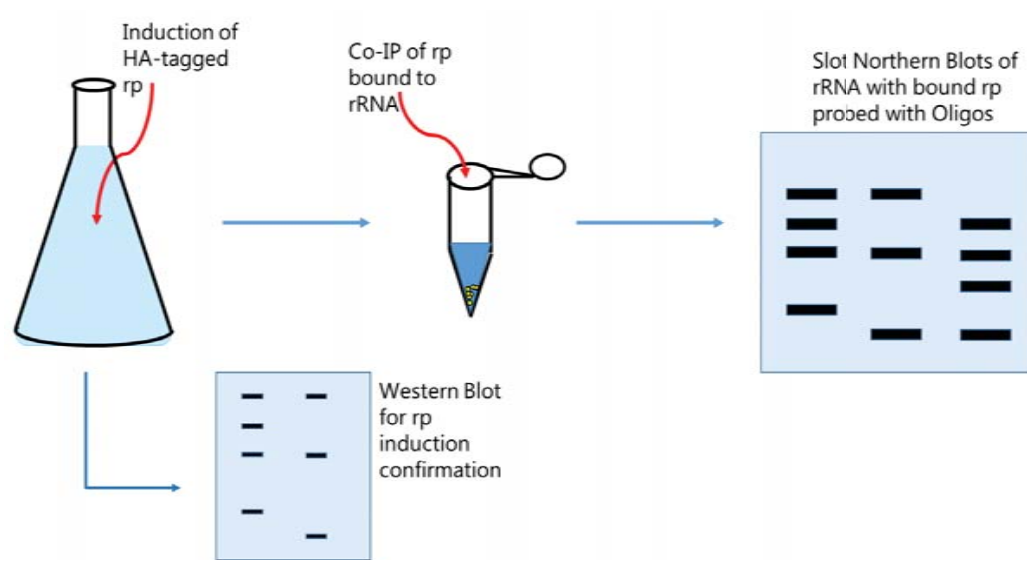
Stepwise cleavage of pre-rRNA and addition of ribosomal proteins



- Binds when whole pre-rRNA is intact; all segments of pre-rRNA immunoprecipitated with the green protein
- Binds after A cleavage; only pre-rRNA segments between A and 3' end immunoprecipitated with the blue protein
- Binds after A, B and C cleavages; only pre-rRNA segments between C and 3' end immunoprecipitated with the red protein

To investigate the correlation of ribosomal protein binding to maturing rRNA to rRNA cleavage points, we have developed a method to study this correlation *in vivo*.

METHODS



RESULTS

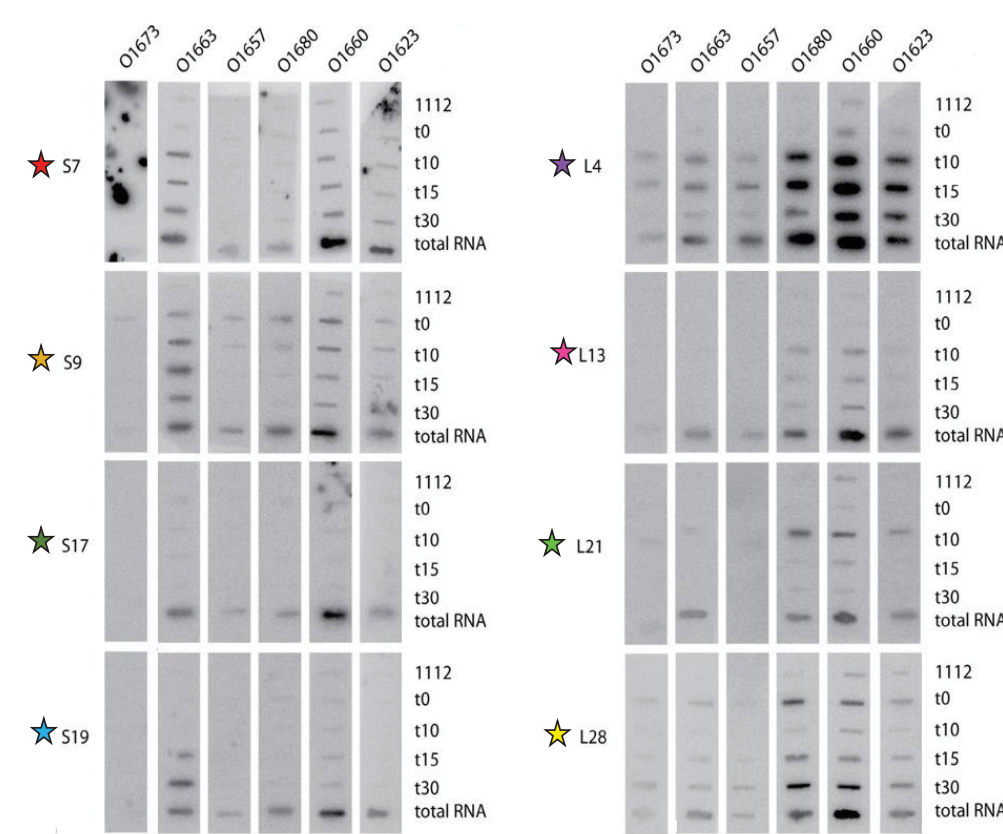


FIGURE 2 (ABOVE): Slot Northern images of Co-IP'ed proteins bound to pre-rRNA, tagged with Oligo probes corresponding to different segments on the rRNA. 1112=Negative control, containing no synthesized protein. Total RNA=Positive control, containing all the ribosomal proteins 30 minutes after induction.

FIGURE 3 (BELOW): Quantitative graphical representation of Slot Northern results depicted in Figure 2 above. Example of rp L4.

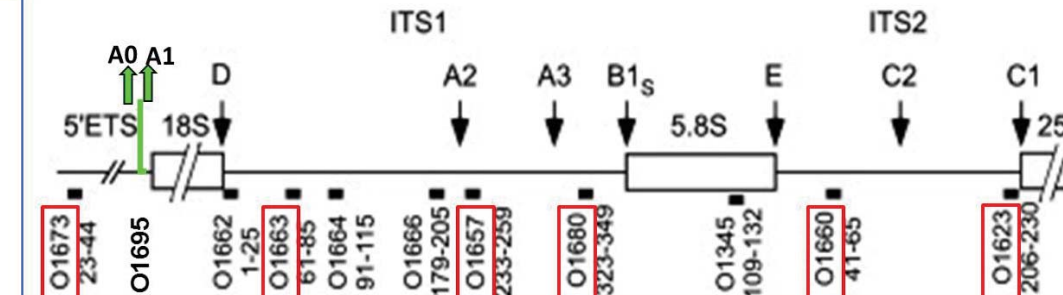
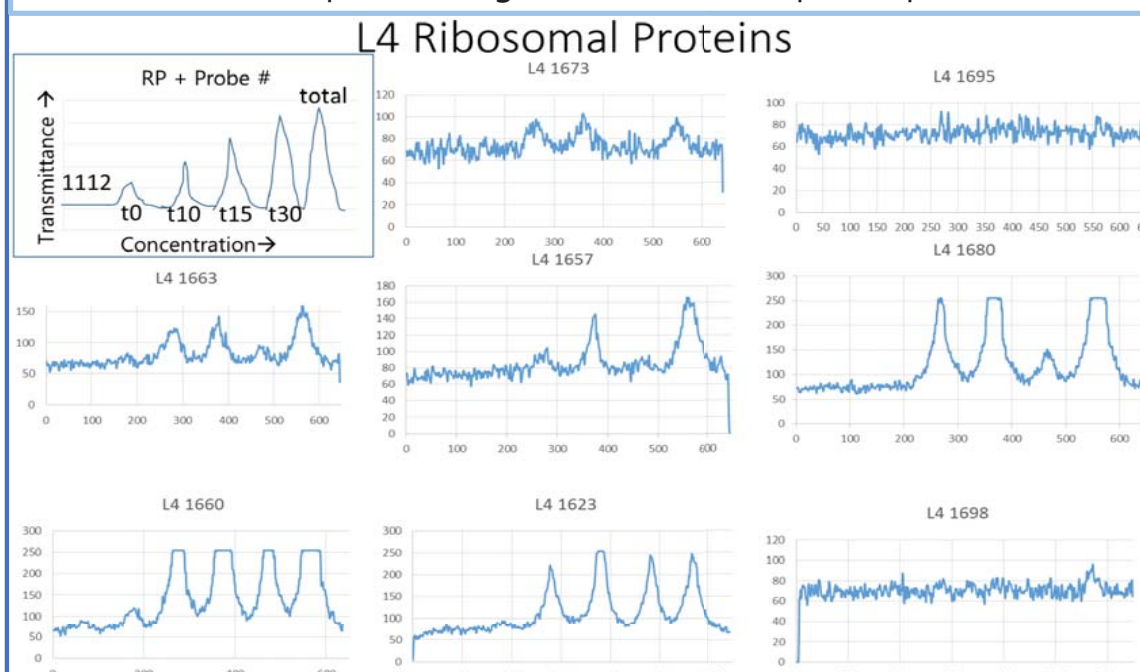
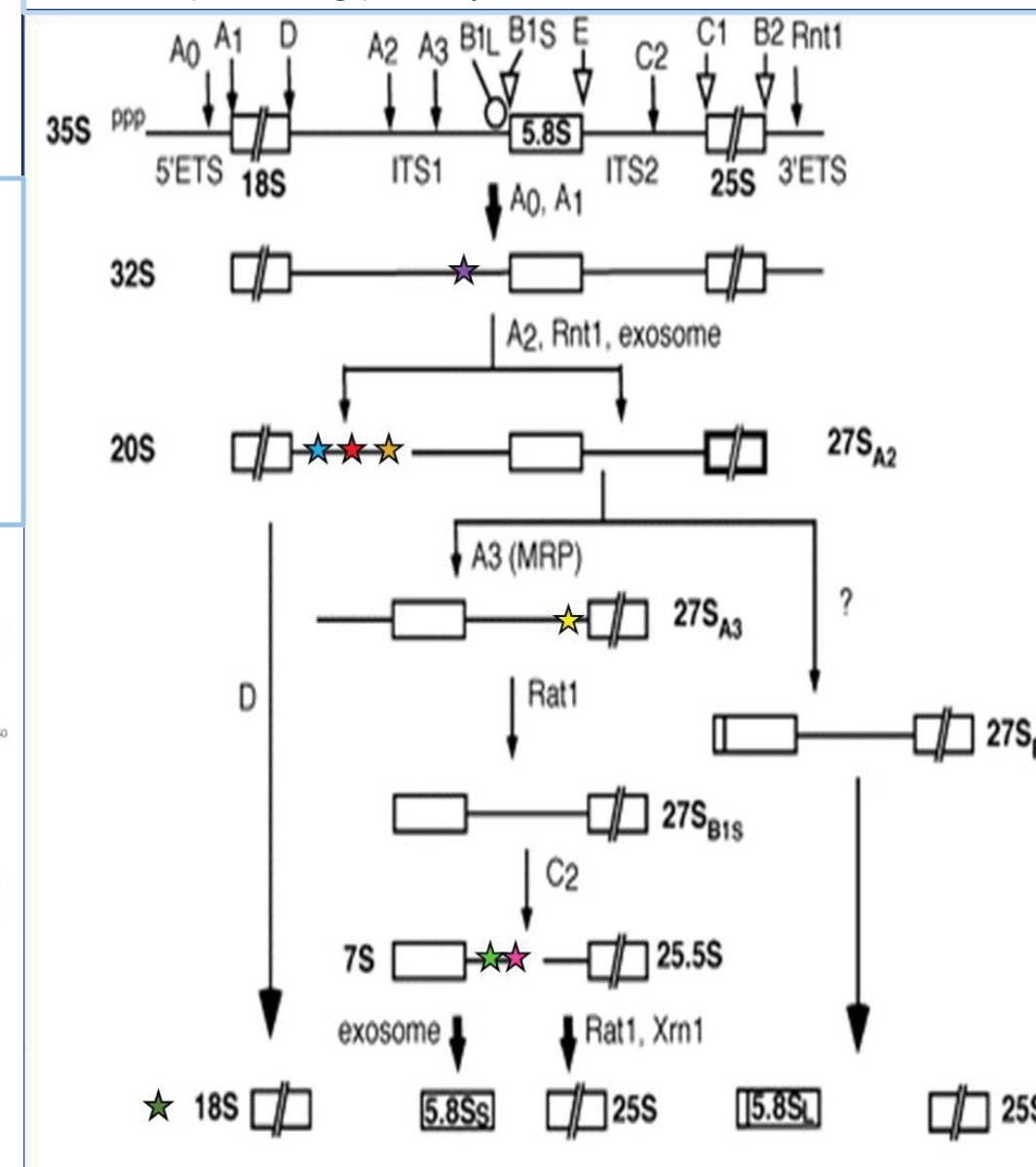


FIGURE 4 (ABOVE): Letters denote order of cleavage sites of maturing RNA. Numbers denote oligo probe sites corresponding to specific sequences on the transcript. Probes boxed in red show the probes used in this project. Image from "RNase MRP is required for entry of 35S precursor rRNA into the canonical processing pathway"-Lindahl et al.

FIGURE 5 (BELOW): Image of rRNA maturation process. Stars indicate estimated binding site locations of tested rps. Image from "RNase MRP is required for entry of 35S precursor rRNA into the canonical processing pathway"-Lindahl et al.



CONCLUSIONS

- Devised a reliable method for studying ribosomal protein binding to ribosomal RNA during maturation.
- Quantification of qualitative data gives normalized standard for comparing and interpreting ribosomal protein binding Slot Northern signals

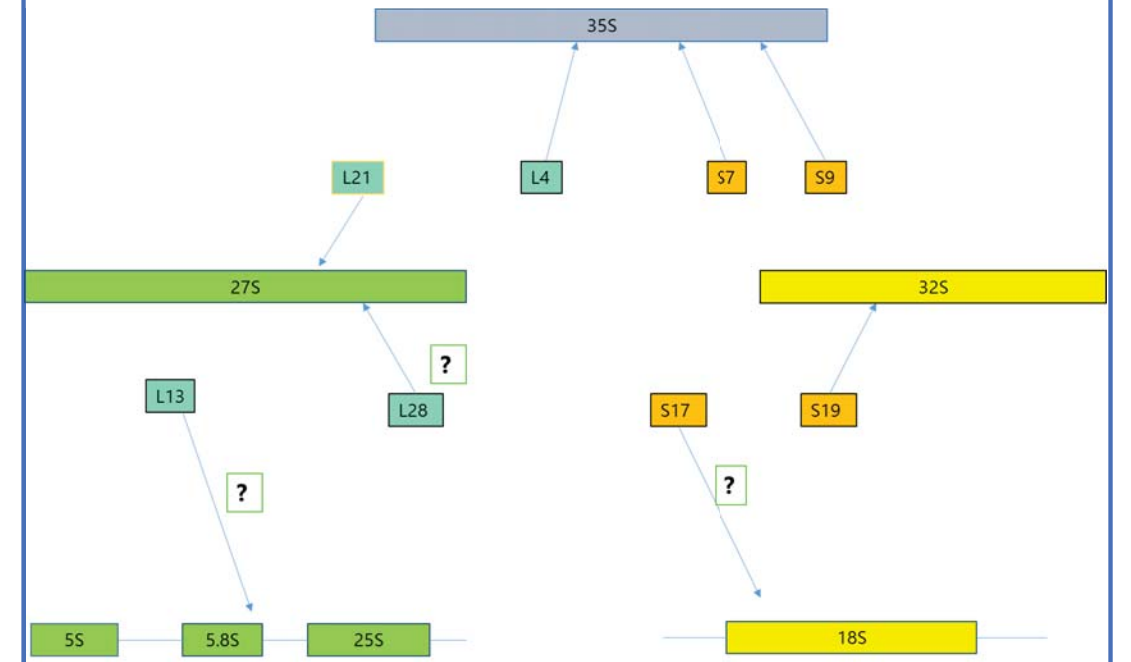


FIGURE 6 (ABOVE): Assembly map depicting interpretation of results. Yellow segments denote pre- and mature rRNA destined for SSU; green segments denote pre- and mature rRNA destined for LSU. Blue rectangles represent LSU rps; orange rectangles represent SSU rps. Question marks are preliminary estimations.

FUTURE DIRECTIONS

- Focus pool of studied proteins to suspected primary binding proteins
- Perform biological repeats for confirmation

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